RayBio® Label-Based (L-Series) Human Obesity Array Membrane Kit

Patent Pending Technology
User Manual (Jan 1, 2022)

For the simultaneous detection of the relative expression of 182 Human proteins in serum, plasma, cell culture supernatants, cell/tissue lysates or other body fluids.

L-Series Human Obesity Array, Membrane
AAH-BLM-ADI-2 (2 Sample Kit)
AAH-BLM-ADI-4 (4 Sample Kit)

Please read manual carefully before starting experiment

RayBiotech
Empowering your proteomics

Your Provider of Excellent Protein Array Systems and Services

---

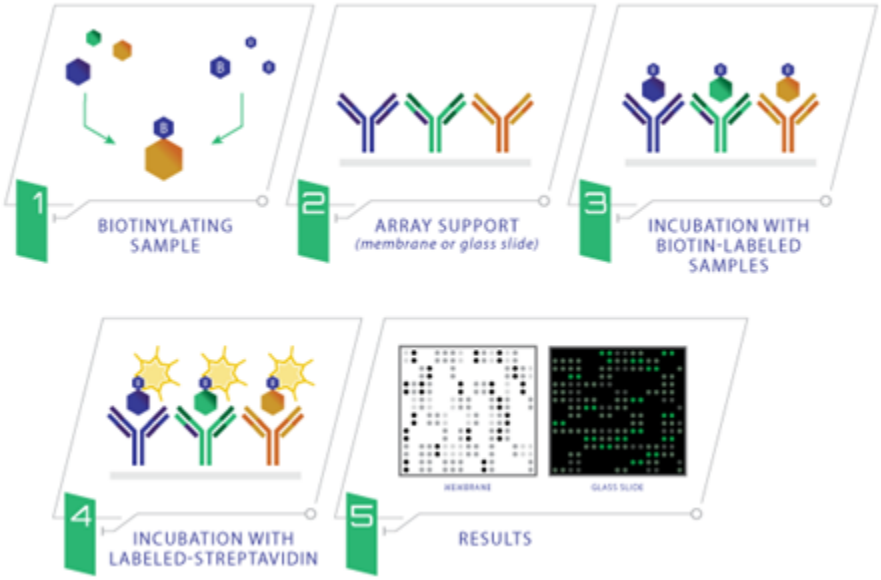
Tel: (Toll Free) 1-888-494-8555 or +1-770-729-2992; Fax: +1-770-206-2393;
Website: www.raybiotech.com Email: info@raybiotech.com

TABLE OF CONTENTS

I. Introduction

Combining direct antigen-labeling technology with our vast library of array-validated antibodies, RayBiotech has created the largest commercially available antibody array to date. With the L-Series high density array platform, researchers can now detect thousands of proteins simultaneously, obtaining a broad, panoramic view of protein expression. Our newly expanded panel includes a wide variety of metabolic enzymes, structural proteins, epigenetic markers, neuroregulatory factors, in addition to our popular list of cytokines, growth factors, receptors, adipokines, proteases, and signaling proteins. Available on both glass slide and membrane formats, this array is ideally suited for biomarker discovery studies and exploratory screens.

The first step in using the RayBio® L-Series Antibody Array is to biotinylate the primary amine groups of the proteins in your sample (sera or plasma, cell culture supernatants, cell lysates or tissue lysates). The membrane arrays are then blocked, similar as a Western blot, and the biotin-labeled sample is added onto the membrane array which is pre-printed with capture antibodies and incubated to allow for interaction of target proteins. After incubation with HRP-Conjugated Streptavidin, the signals can be visualized by chemiluminescence.



II. Materials Provided

A. Storage Recommendations

Upon receipt, Box 1 should be stored at -20°C and Box 2 should be stored at 4°C. The kit must be used within 6 months from the date of shipment. After initial use, Blocking Buffer, Stop Solution, HRP-Conjugated Streptavidin, Detection Buffers C and D should be stored at 4°C to avoid repeated freeze-thaw cycles (may be stored for up to 3 months, Labeling Reagent, Item B should be fresh preparation before use). The Array Membrane should be kept at -20°C and avoid repeated freeze-thaw cycles (may be stored for up to 6 months).

Box 1 (store at -20°C):

ITEM	DESCRIPTION	2 MEMBRANE KIT	4 MEMBRANE KIT
B	Labeling Reagent	1 vial	2 vials
D	Stop Solution	1 vial (50 µl)	1 vial (50 µl)
E	L-series Antibody Array Membranes	2 membranes	4 membranes
F	4X Blocking Buffer	1 bottle (30 ml)	1 bottle (30 ml)
I	500X HRP-Conjugated Streptavidin Concentrate	1 vial (100 µl)	1 vial (100 µl)
K	Detection Buffer C	1 bottle (10 ml)	2 bottles (10 ml)
L	Detection Buffer D	1 bottle (10 ml)	2 bottles (10 ml)
Other Kit Components: Plastic Sheets			

Box 2 (store at 4°C):

ITEM	DESCRIPTION	2 MEMBRANE KIT	4 MEMBRANE KIT
G	20X Wash Buffer 1 Concentrate	1 bottle (30 ml)	1 bottle (30 ml)
H	20X Wash Buffer 2 Concentrate	1 bottle (30 ml)	1 bottle (30 ml)
	Labeling Buffer	1 bottle (30 ml)	2 bottles (30 ml/ea)
J-2	Spin Columns	4 columns	8 columns
N/A	Plastic Incubation Trays (w/lid)	2 trays	4 trays
N/A	2X Lysis Buffer	1 bottle (10 ml)	1 bottle (10 ml)

B. Additional Materials Required

- 2-5 ml tube, small plastic or glass containers
- 15 ml conical collection tubes
- Orbital shaker or oscillating rocker
- Kodak X-Omat™ AR film (REF 165 1454) and film processor or Chemiluminescence imaging system
- Pipettors, pipette tips and other common lab consumables
- Eppendorf tube

III. Overview and General Considerations

A. Preparation and Storage of Samples

1. Preparation of Cell Culture Supernatants
    1. Seed cells at a density of $1x10^6$ cells in 100 mm tissue culture dishes.*
    2. Culture cells in complete culture medium for ~24-48 hours.**
    3. Replenish with serum-free or low-serum medium such as 0.2% FCS/FBS serum, and then incubate cells again for ~48 hours.**,+
    4. To collect supernatants, centrifuge at 1,000 x g for 10 minutes and store as less than or equal 1 ml aliquots at -80°C until needed.
    5. If you want to use cell mass for inter-sample normalization, measure the total wet weight of cultured cells in the pellet and/or culture dish. You may then normalize between arrays by dividing densitometry signals by total cell mass (i.e., express results as the relative amount of protein expressed/mg total cell mass). Or you can normalize between arrays by determining cell lysate concentration using a total protein assay (BCA Protein Assay Kit, Pierce, Prod #: 23227).

        **The density of cells per dish used is dependent on the cell type. More or less cells may be required.*

        ***Optimal culture time may vary and will depend on the cell line, treatment conditions and other factors.*

+Bovine serum proteins produce detectable signals on the RayBio® L-Series Array in media containing serum concentrations as low as 0.2%. When testing serum-containing media, we strongly recommend testing an uncultured media blank for comparison with sample results.

2. Extracting Protein from Cells
    1. Centrifuging Cells
        a. Adherent Cells:
            i. Remove supernatant from cell culture and wash cells gently twice with cold 1X PBS taking care not to disturb cell layer.
            ii. Add enough cold 1X PBS to cover cell layer and use cell scraper to detach cells.
        b. Cells in Suspension: Pellet the cells by centrifuging using a microcentrifuge at 1500 rpm for 10 minutes.
    2. Make sure to remove any remaining PBS before adding 1X Cell Lysis Buffer (2X Cell Lysis Buffer should be diluted 2-fold with ddH_2O). Solubilize the cells at $2x10^7$ cells/ml in 1X Cell Lysis Buffer.
    3. Pipette up and down to resuspend cells and rock the lysates gently at 2-8°C for 30 minutes. Transfer extracts to microfuge tubes and centrifuge at 13,000 rpm for 10 minutes at 2-8°C.

        Note: If the lysates appear to be cloudy, transfer the lysates to a clean tube, centrifuge again at 13,000 rpm for 20 minutes at 2-8°C. If the lysates are still not clear, store them at -20°C for 20 minutes. Remove from the freezer and immediately centrifuge at 13,000 rpm for 20 minutes at 2-8°C.
    4. Transfer lysates to a clean tube. Determining cell lysate concentrations using a total protein assay (BCA Protein Assay Kit, Pierce, Prod# 23227). Aliquot the lysates and store at -80°C.
3. Extracting Protein from Crude Tissue
    1. Transfer approximate 100 mg crude tissue into a tube with 1 ml 1X Cell Lysis Buffer (2X Cell Lysis Buffer should be diluted 2-fold with ddH_2O).
    2. Homogenize the tissue according to homogenizer manufacturer instructions.

3. Transfer extracts to microcentrifuge tubes and centrifuge for 20 minutes at 13,000 rpm (4°C).

   Note: If the supernatant appears to be cloudy, transfer the supernatants to a clean tube, centrifuge again at 13,000 rpm for 20 minutes at 2-8°C. If the supernatant is still not clear, store the lysate at -20°C for 20 minutes. Remove from the freezer, immediately centrifuge at 13,000 rpm for 20 minutes at 2-8°C.

4. Transfer supernatant to a clean tube and store at -80°C.

4. Determine the total protein concentration
   For optimal biotin labeling, it is necessary to determine the protein concentration in the cell/tissue lysate. We recommended using a BCA total protein assay (e.g., Pierce, Catalog # 23227).

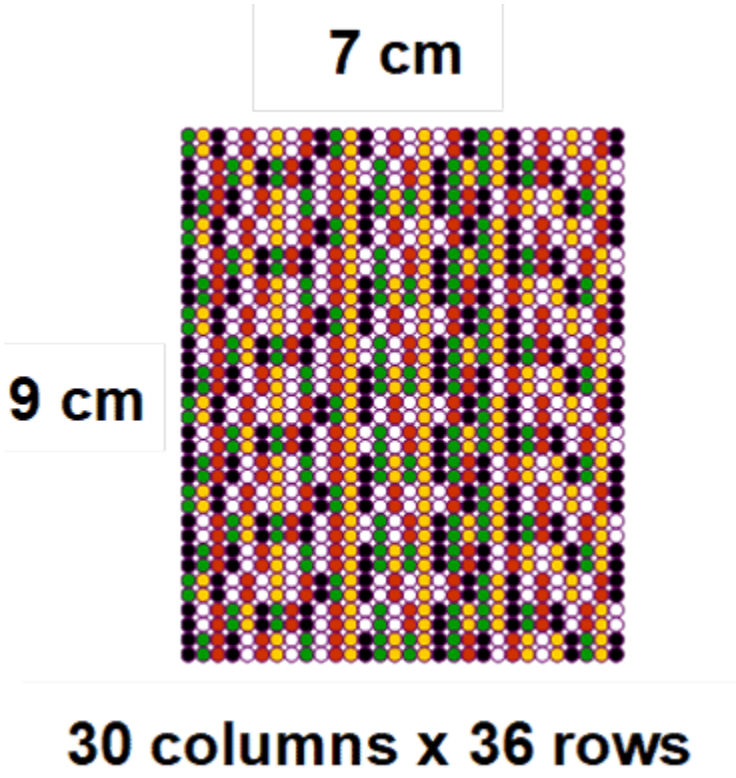
B. Handling the Array Membranes

- Always use forceps to handle membranes and grip the membranes by the edges only.
- Never allow membranes to dry during the experiment.
- Avoid touching membranes with hands or any sharp tools.

C. Incubations of Antibody Array

- Completely cover membranes with sample or buffer during incubation and cover the Plastic Incubation Tray with the lid to avoid drying.
- Avoid foaming during incubation steps.
- Perform all incubation and wash steps under gentle rotation.
- Several incubation steps such as step 3 (sample incubation) or step 7 (HRP-Conjugated Streptavidin incubation) may be done at 4°C overnight.

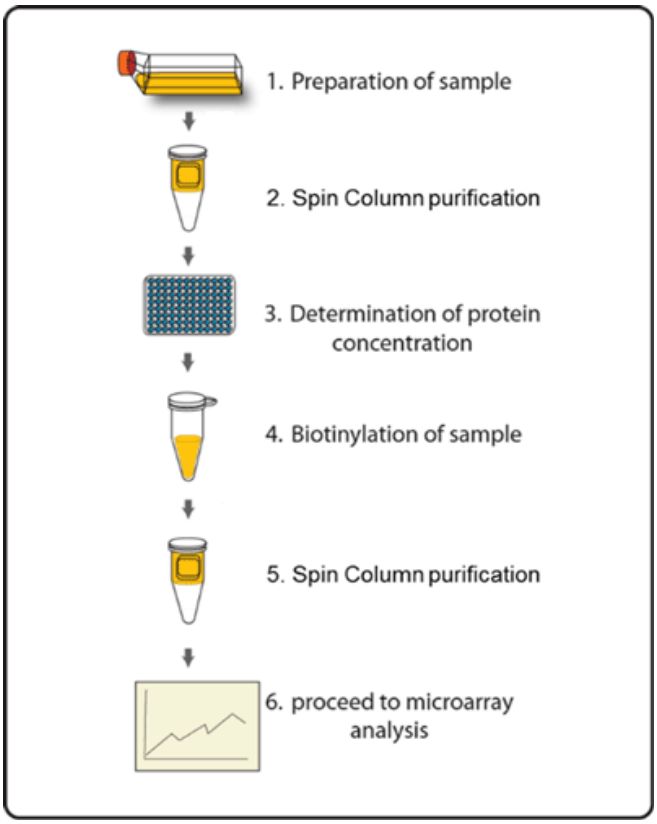
D. Layout of Array Membrane



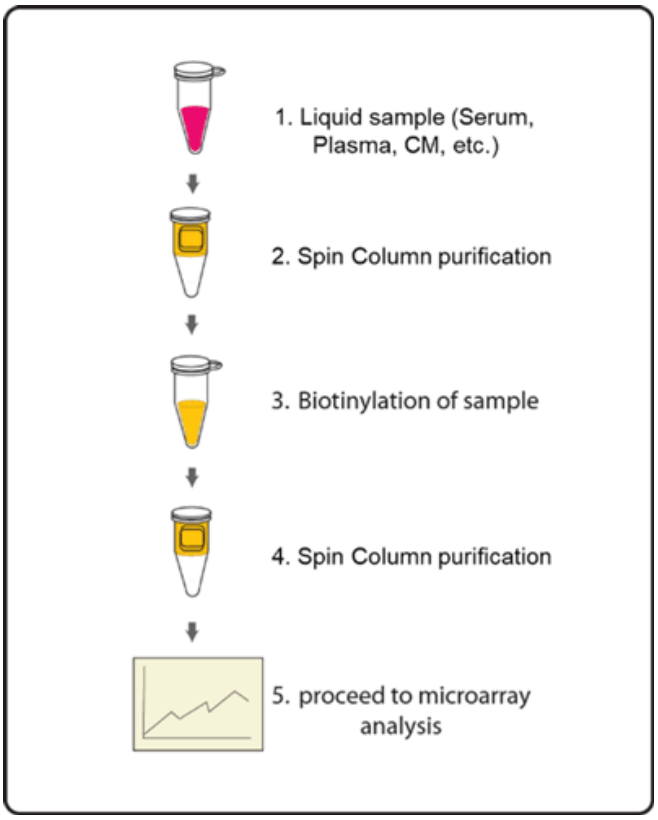
IV. Protocol

Assay Diagram

1. Cell/tissue lysates



2. Serum, plasma, or Cell culture supernatants



A. Sample purification

Note: This step removes low molecular weight amine derivatives or unwanted buffer from samples to ensure quality biotinylation in Steps 5-7.

1. Twist to remove the bottom plug of the Spin Column and loosen the cap (do not remove).
2. Place the Spin column into a 15 ml conical collection tube, centrifuge at 1,000 x g for 3 minutes to remove the storage buffer. Discard the flow-through.
3. Wash the column three times with 1 ml labeling buffer each, centrifuge 1,000 x g for 3 minutes to remove the flow-through. Blot the bottom of the column to remove excess liquid, and transfer device to a new collection tube.
4. Apply sample on top of the resin within the next few minutes. Centrifuge at

1,000 x g for 3 minutes to collect the flow-through that contains sample. The recommended sample dilution as following:

- *Cell culture supernatant: 600 µl neat supernatant*
- *Serum/Plasma: 10 µl serum/plasma in 600 µl Labeling Buffer*
- *Cell/tissue lysate: 100 µg lysate in 500 µl Labeling Buffer*

Note: The maximal sample volume is 700 µl for each Spin Column. Do not load over 700 µl of sample into a Spin Column.

B. Biotin-Labeling the Sample

Note: Amines (e.g., Tris, glycine) and azides quench the biotinylation reaction. Avoid contaminating samples with these chemicals prior to biotinylation.

5. Immediately before use, prepare the Labeling Reagent. Briefly spin down the Labeling Reagent tube (Item B). Add 100 µl Labeling Buffer into the tube, then pipette up and down or vortex slightly to dissolve the lyophilized reagent.
6. Add Labeling Reagent to the sample tube. Incubate the reaction solution at RT with gentle rocking or shaking for 30 min. Mix the reaction solution by gently tapping the tube every 5 minutes.
    a. For labeling cell culture supernatants: Add 10 µl of Labeling Reagent into the sample tube (for 600 µl supernatant).
    b. For labeling serum or plasma: Add 10 µl of Labeling Reagent into the sample tube (for 10 µl serum/plasma *in 600 µl labeling buffer*).
    c. For labeling cell or tissue lysates: Add 5 µl of 1X Labeling Reagent into the sample tube (for 100 µg lysate *in 500 µl labeling buffer*).
    d. For all other body fluid: Add 2 µl of Labeling Reagent Solution per 100 µg sample to be labelled.

    Note: The addition of Labeling Reagent volume is based upon the sample amount used in Step 4. If more or less amount sample is labelled, adjust this volume proportionally.
7. Add 5 µl Stop Solution (Item D) to each sample tube. Using a new spin column, repeat Steps 1-4 of section A. Sample Purification to remove the

excess non-reacted biotin reagent from each sample.

Note: Biotinylated samples can be stored at -20°C or -80°C until you are ready to proceed with the assay.

C. Blocking and Incubations

8. Place each membrane printed side up into a Plastic Incubation Tray (provided). 1 membrane per tray.

   Note: The printed membrane will have a "-" mark in the upper left corner of the membrane.

   Note: Up to 4 membranes can be incubated together within one tray with proportional amount of reaction buffer. Rotate the membrane sequence at least once during sample incubation if more than one membrane is incubated in one tray.

9. Dilute 4X Blocking Buffer (Item F) with deionized or distilled water to prepare the 1X Blocking Buffer. Add 6 ml of 1X Blocking Buffer to each membrane and cover with the lid. Incubate at room temperature with gentle shaking for 1 hour.

10. Aspirate the Blocking Buffer from each tray. Add 6 ml of diluted sample onto each membrane and cover with the lid. Incubate at room temperature with gentle shaking for 2 hours.

    Note: It is recommended to use 10-20 folds diluted biotin-labeled culture supernatant, 10-20 folds diluted biotin-labeled serum/plasma, 100 folds diluted biotin-labeled cell/tissue lysate, or 10-20 folds for other body fluid. Dilute sample using 1X Blocking Buffer. The optimal concentration of sample used will depend on the abundance of target proteins. The samples can be concentrated if the overall signals are too weak. If the overall signals are too strong, the sample can be diluted further.

    Note: Incubation may be done at room temperature with gentle shaking for 2 hours or overnight at 4°C.

11. Dilute 20X Wash Buffer 1 (Item G) with deionized or distilled water to prepare the 1X Wash Buffer 1. Aspirate the samples from each tray and then wash by adding 20 ml of 1X Wash Buffer I at room temperature with gentle shaking (5 min per wash). Repeat the wash 2 more times for a total of 3 washes.

12. Aspirate the 1X Wash Buffer 1 from each tray. Dilute 20X Wash Buffer 2 (Item H) with deionized or distilled water to prepare the 1X Wash Buffer 2. Wash 3 times with 20 ml of 1X Wash Buffer 2 at room temperature with gentle shaking.
13. Aspirate the 1X Wash Buffer 2 from each tray.
14. Prepare the HRP-Conjugated Streptavidin. Briefly spin down the tube containing the 500X HRP-Conjugated Streptavidin (Item I) immediately before use. Dilute the 500X HRP-Conjugated Streptavidin with 1X Blocking Buffer to prepare the 1X HRP-Conjugated Streptavidin. Pipette up and down to mix gently. Add 6 ml of 1X HRP-Conjugated Streptavidin to each membrane.

    Note: Ensure that the vial containing the 500X HRP-Conjugated Streptavidin is mixed well before use, as precipitation can form during storage.
15. Incubate at room temperature with gentle shaking for 2 hours.

    Note: incubation may be done overnight at 4°C.
16. Wash as directed in steps 11 through 13.

D. Detection

Note: Do not let the membrane dry out during detection. The detection process must be completed within 40 minutes without stopping.

17. For detection of 2 membranes, add 4.2 ml of Detection Buffer C and 4.2 ml of Detection buffer D into a tube and mix both solutions. Drain off excess wash buffer. Place membrane antibody side up (There is a "-" symbol on the top left corner of each membrane) on a clean plastic plate or its cover (provided in the kit). Pipette 4 ml of the mixed Detection Buffers onto each membrane and incubate at room temperature for 2 minutes with gentle shaking. Ensure that the detection mixture is evenly covering the membrane without any air bubbles.
18. Gently place the membrane with forceps (antibody side up) on a plastic sheet (provided) and cover the membrane with another plastic sheet. Gently smooth out any air bubbles. Avoid using pressure on the membrane. Work as quickly as possible.
19. The signal can be detected directly from the membrane using a chemiluminescence imaging system or by exposing the array to x-ray film (we recommend using Kodak X-Omat™ AR film) with subsequent development.

Expose the membranes for 40 seconds. Then re-expose the film according to the intensity of signals. If the signals are too strong (background too high), reduce the exposure time (e.g., 5-30 seconds). If the signals are too weak, increase the exposure time (e.g., 5-20 min or overnight) or re-incubate membranes overnight with 1X HRP-Conjugated Streptavidin, and repeat detection on the second day.

20. Save membranes at -20°C to -80°C for future reference.

V. Antibody Array Map

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1	POS1	POS2	POS3	Blank	Blank	NEG	NEG	Blank	1	2	3	4	5	Blank	Blank	Blank	Blank	Blank	Blank	Blank	NEG	NEG	Blank	6	7	8	9	10	11	12
2	POS1	POS2	POS3	Blank	Blank	NEG	NEG	Blank	1	2	3	4	5	Blank	Blank	Blank	Blank	Blank	Blank	Blank	NEG	NEG	Blank	6	7	8	9	10	11	12
3	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	13	14	15	16	17	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	18	19	20	21	22	23	24
4	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	13	14	15	16	17	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	18	19	20	21	22	23	24
5	25	26	27	28	29	30	31	32	33	34	35	36	37	Blank	Blank	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52
6	25	26	27	28	29	30	31	32	33	34	35	36	37	Blank	Blank	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52
7	53	54	55	56	57	58	59	60	61	62	63	64	65	Blank	Blank	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80
8	53	54	55	56	57	58	59	60	61	62	63	64	65	Blank	Blank	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80
9	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	81	82	83	84	85	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	86	87	88	89	90	91	92
10	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	81	82	83	84	85	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	86	87	88	89	90	91	92
11	Blank	Blank	Blank	Blank	Blank	NEG	NEG	Blank	93	94	95	96	97	Blank	Blank	Blank	Blank	Blank	Blank	Blank	NEG	NEG	Blank	98	99	100	101	102	103	104
12	Blank	Blank	Blank	Blank	Blank	NEG	NEG	Blank	93	94	95	96	97	Blank	Blank	Blank	Blank	Blank	Blank	Blank	NEG	NEG	Blank	98	99	100	101	102	103	104
13	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	105	106	107	108	109	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	110	111	112	113	114	115	116
14	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	105	106	107	108	109	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	110	111	112	113	114	115	116
15	117	118	119	120	121	122	123	124	125	126	127	128	129	Blank	Blank	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144
16	117	118	119	120	121	122	123	124	125	126	127	128	129	Blank	Blank	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144
17	145	146	147	148	149	150	151	152	153	154	155	156	157	Blank	Blank	158	159	160	161	162	163	164	165	166	167	168	169	170	171	172
18	145	146	147	148	149	150	151	152	153	154	155	156	157	Blank	Blank	158	159	160	161	162	163	164	165	166	167	168	169	170	171	172
19	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	173	174	175	176	177	Blank	Blank	178	179	180	181	182	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
20	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	173	174	175	176	177	Blank	Blank	178	179	180	181	182	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
21	Blank	Blank	Blank	Blank	Blank	NEG	NEG	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	NEG	NEG	Blank	Blank	POS3	POS2	POS1
22	Blank	Blank	Blank	Blank	Blank	NEG	NEG	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	NEG	NEG	Blank	Blank	POS3	POS2	POS1

VI. Antibody Array Target List

Number	Name	Number	Name	Number	Name	Number	Name
1	ACE	51	Epiregulin	101	Leptin	151	S100 A8/A9
2	ACE-2	52	E-selectin	102	Leptin R	152	S100 A10
3	ACTH	53	Endothelin-1	103	Luteinizing Hormone	153	SAA1
4	Perilipin-2	54	FABP4	104	LIF	154	SDF-1
5	Adiponectin	55	FAM3B	105	LOX-1	155	SEMA3A
6	Adipsin	56	FAS	106	Lymphotactin	156	Serotonin
7	AgRP	57	FGF-10	107	MCP-1	157	Syndecan-3
8	AMPKa1	58	FGF-6	108	MCP-3	158	TACE
9	Amylin	59	FSH	109	M-CSF	159	uPA
10	Angiopoietin-1	60	Galectin-1	110	MIF	160	TECK
11	Angiopoietin-2	61	Growth Hormone	111	MIP-1 alpha	161	TGF alpha
12	Angiotensinogen	62	Ghrelin	112	MIP-1 beta	162	TGF beta 1
13	ANGPTL7	63	GITR	113	MIP-3 beta	163	Thrombospondin-1
14	ANGPTL1	64	GITR Ligand	114	MMP-2	164	Thrombospondin-2
15	ANGPTL2	65	GLP-1	115	MMP-9	165	Thrombospondin-4
16	ANGPTL3	66	Glucagon	116	MMP-11	166	TIMP-1
17	ANGPTL4	67	Glut1	117	MMP-19	167	TIMP-2
18	APJ	68	Glut2	118	MSHa	168	TIMP-3
19	ApoB	69	Glut3	119	MSP alpha	169	TIMP-4
20	ApoE	70	Glut5	120	Myostatin	170	Coagulation Factor III
21	Axl	71	GPX1	121	NAIP	171	TLR2
22	BDNF	72	GPX3	122	NeuroD1	172	TLR4
23	bFGF	73	GRO alpha	123	Neurophilin-2	173	TNF alpha
24	BMP-2	74	HCC-4	124	NGFR	174	TNF RI
25	BMP-3	75	HGF	125	NPY	175	TNF RII
26	BMP-3b	76	11-beta-HSD1	126	GPR39	176	TSG-6
27	BMP-4	77	ICAM-1	127	Orexin-A	177	TSH
28	BMP-5	78	IFN-gamma	128	Orexin-B	178	Vaspin
29	BMP-6	79	IGF-1	129	Oncostatin M	179	VCAM-1
30	BMP-7	80	IGF-1 R	130	Osteocalcin	180	VEGF
31	BMP-8	81	IGFBP-1	131	Osteonectin	181	Visfatin
32	BMP-15	82	IGFBP-2	132	Osteoprotegerin	182	XEDAR
33	BMPR-IA	83	IGFBP-3	133	PARC		
34	BMPR-IB	84	IGF-2	134	PDGF-BB		
35	BMPR-II	85	IL-1 R1	135	PDGF-AA		
36	beta-NGF	86	IL-1 R4	136	PDGF-AB		
37	C3a	87	IL-1 alpha	137	PDGF-C		
38	CART	88	IL-1 beta	138	PDGF-D		
39	4-1BB	89	IL-1 ra	139	Serpin F1		
40	CD36	90	IL-6	140	Pentraxin-3		
41	Clusterin	91	IL-6 R	141	PPARg2		
42	CNTF	92	IL-8	142	Pref-1		
43	C-peptide	93	IL-10	143	Prohibitin		
44	CRP	94	IL-11	144	Prolactin		
45	Cystatin C	95	IL-12	145	PYY		
46	Dtk	96	IL-25	146	RANTES		
47	EGF	97	Relaxin-3	147	RBP4		
48	EGFR	98	INSRR	148	RELM beta		
49	ENA-78	99	Insulin	149	Resistin		
50	Endophin Beta	100	Insulin R	150	S100B		

VII. Interpretation of Results:

A. Explanation of Controls Spots

To obtain optimal results using a chemiluminescence imaging system (UVP BioImaging Systems), it is suggested to try several different exposure times until the best one is determined. Then, by comparing the signal intensities, relative expression levels of the target proteins can be made. The intensities of signals can be quantified by densitometry. There are three Positive Controls (POS1, POS2, POS3) in each array. These are three levels of standardized anti-HRP antibodies, which will produce positive control signals after incubation with HRP-conjugated Streptavidin. With all other variables being equal, the Positive Control intensities will be the same for each sub-array, which allows for inter-array normalization. Antibody affinity to its target varies significantly between antibodies. The intensity detected on the array with each antibody depends on this affinity; therefore, signal intensity comparison can be performed only within the same antibody/antigen system and not between different antibodies. Some arrays may have beta-actin and GAPDH as internal controls, much as "housekeeping" genes or proteins are used to normalize results in PCR or Western blots, respectively.

B. Typical Results

The following figure shows the typical result of this array probed with sample(s).

Sample image

Image not found
https://doc.raybiotech.com/assets/img/l-series/samples/AAH-BLM-ADI.jpg

Note: In the absence of an external standard curve for each protein detected, there is no means of assessing absolute or relative concentrations of different proteins in the same sample using immunoassays. If you wish to obtain quantitative data (i.e., concentrations of the various analytes in your samples), try using our Quantibody® Arrays as a targeted follow-up experiment.

C. Background Subtraction

Once you have obtained densitometry data, it is recommended to subtract the local background and normalize to the Positive Control signals before proceeding to analysis.

D. Normalization of Array Data

To normalize signal intensity data, one sub-array is defined as "reference" to which the other arrays are normalized. This choice is arbitrary. For example, in our Analysis Tool Software (described below), the array represented by data entered in the left-most column each worksheet is the default "reference array."

You can calculate the normalized values as follows:

X(Ny) = X(y) * P1/P(y)

Where:
P1 = mean signal intensity of POS spots on reference array
P(y) = mean signal intensity of POS spots on Array "y"
X(y) = mean signal intensity for spot "X" on Array "y"
X(Ny) = normalized signal intensity for spot "X" on Array "y"

The RayBio® Analysis Tool software is available for use with data obtained using RayBio® Biotin Label-based Antibody Arrays. You can copy and paste your signal intensity data (with and without background) into the Analysis Tool, and it will automatically normalize signal intensities to the Positive Controls.

E. Threshold of Significant Difference

After subtracting background signals and normalization to Positive Controls, comparison of signal intensities between and among array images can be used to determine relative differences in expression levels of each protein between samples or groups.

Any greater than or equal to 1.5-fold increase or less than or equal to 0.65-fold decrease in signal intensity for a single analyte between samples or groups may be considered a measurable and significant difference in expression, provided that both sets of signals are well above background (Mean background + 2 standard deviations, accuracy is around 95%).

VIII. Troubleshooting Guide

Problem	Cause	Recommendation
Weak Signal	Taking too much time for detection	The whole detection process must be completed within 30 min
	Film developer does not work properly	Fix film developer
	Did not mix HRP-Streptavidin well before use	Mix tube containing HRP-Conjugated Streptavidin well before use since precipitates may form during storage
	Sample is too diluted	Increase sample concentration
	Labeling reagent does not function well	Labeling reagent needs to be saved in -20°C and avoid freeze thaw cycle. Always use fresh labeling reagent for sample labelling.
	Other	Check if there were any contamination with any solution containing amines in biotin-labeling step
		Slightly increase HRP concentrations
		Work as quickly as possible after mix Detection Buffer C and D
Uneven signal	Bubble formed during incubation	Remove bubbles during incubation
	Membranes were not completely covered with solution	Completely cover membranes with solution
	Insufficient wash	Use more stringent wash
High background	Exposure time is too long	Decrease exposure time
	Membranes dry out during experiment	Completely cover membranes with solution during experiment. Cover tray with lid.
	Sample is too concentrated	Dilute sample

IX. Selected References

Christina Scheel et all., *Paracrine and Autocrine Signals Induce and Maintain Mesenchymal and Stem Cell States in the Breast.* Cell. 2011;145, 926-940.

Lin Y, Huang R, Chen L, et al., *Profiling of cytokine expression by biotin-labeled-based protein arrays.* Proteomics. 2003, 3: 1750-1757.

Huang R, Jiang W, Yang J, et al., *A Biotin Label-based Antibody Array for High-content Profiling of Protein Expression.* Cancer Genomics Proteomics. 2010; 7(3):129-141.

Liu T, Xue R, Dong L, et al., *Rapid determination of serological cytokine biomarkers for hepatitis B-virus-related hepatocellulare carcinoma using antibody arrays.* Acta Biochim Biophys Sin. 2011; 43(1):45-51.

Cui J, Chen Y, Chou W-C, et al., *An integrated transcriptomic and computational analysis for biomarker identification in gastric cancer.* Nucl Acids Res. 2011; 39(4):1197-1207.

Jun Zhong et all., *Temporal Profiling of the Secretome during Adipogenesis in Humans.* Journal of Proteome Research. 2010, 9, 5228-5238.

Chowdury UR, Madden BJ, Charlesworth MC, Fautsch MP., *Proteomic Analysis of Human Aqueous Humor.* Invest Ophthalmol Visual Sci. 2010; 51(10):4921-4931.

Wei Y, Cui C, Lainscak M, et al., *Type-specific dysregulation of matrix metalloproteinases and their tissue inhibitors in end-stage heart failure patients: relationship between MMP-10 andLV remodeling.* J Cell Mol Med. 2011; 15(4):773-782.

Kuranda K, Berthon C, LepÃªtre F, et al., *Expression of CD34 in hematopoietic cancer cell lines reflects tightly regulated stem/progenitor-like state.* J Cell Biochem. 2011; 112(5):1277-1285.

Toh HC, Wang W-W, Chia WK, et al., *Clinical Benefit of Allogenic Melanoma Cell Lysate-Pulsed Autologous Dendritic Cell Vaccine in MAGE-Positive Colorectal Cancer Patients.* Clin Chem Res. 2009; 15:7726-7736.

Zhen Hou, *Cytokine array analysis of peritoneal fluid between women with endometriosis of different stages and those without endometriosi.* Biomarkers. 2009;14(8): 604-618.

Yao Liang Tang, et al., *Hypoxic Preconditioning Enhances the Benefit of Cardiac Progenitor Cell Therapy for Treatment of Myocardial Infarction by Inducing CXCR4.*

Circ Res. 2009;109:197723.

RayBio® L-series Antibody Arrays are patent-pending technology developed by RayBiotech.

This product is intended for research only and is not to be used for clinical diagnosis. Our produces may not be resold, modified for resale, or used to manufacture commercial products without written approval by RayBiotech, Inc.

Under no circumstances shall RayBiotech be liable for any damages arising out of the use of the materials.

Products are guaranteed for six months from the date of shipment when handled and stored properly. In the event of any defect in quality or merchantability, RayBiotech's liability to buyer for any claim relating to products shall be limited to replacement or refund of the purchase price.

This product is for research use only.